Patterning Antigens with Near-Field Optics

Matthew Kiok
Chemistry, Tulane University

NNIN REU Site: Cornell NanoScale Science and Technology Facility, Cornell University, Ithaca, NY
NNIN REU Principal Investigator: Professor Harold Craighead, Applied and Engineering Physics, Cornell University
NNIN REU Mentor: Dr. Christopher Kelly, Applied and Engineering Physics, Cornell University
Contact: mkiok@tulane.edu, hgc1@cornell.edu, ck462@cornell.edu

Abstract:

The membrane of a cell is a constantly flowing sea of lipids stacked together in a bilayer. Gaining a better understanding of the diffusion of lipids across the surface can be achieved by modeling with supported lipid bilayers (SLBs) that have incorporated ultraviolet (UV) polymerizable and fluorescent lipids. The goal of this project was to fabricate planar, microscale patterned substrates for creation of SLBs that would be observed with optical fluorescence techniques. Fused silica wafers were patterned by contact lithography, etched by reactive ion etching, coated with a thin film of aluminum by a metal evaporator, and processed by wet chemical liftoff, resulting in a smooth surface with aluminum lying flush with the glass surface. An SLB was constructed on the surface and the diffusion coefficient calculated by fluorescence recovery after photobleaching (FRAP). Future developments include improving the smoothness of the glass substrate, promoting SLB formation with polymerizable lipids, and working with patterns at the nanoscale. After optimization, these techniques can be applied to observe fluorescently tagged antigen-antibody-receptor complexes on the membrane surface and to better understand the cell membrane signaling cascade process.

Introduction:

The interaction between antigens, antibodies, and cellular receptors is a phenomenon not easily examined at high resolution through conventional optics. The use of fluorescent molecules to tag structures of interest permits the observation of their bulk diffusion with a fluorescence microscope. Supported lipid bilayers are used to model cell membranes and can easily be customized to simulate a variety of membrane types. Fluorescence recovery after photobleaching (FRAP) is employed to determine the diffusion coefficient for a particular lipid composition. The calculated diffusion coefficient is related to the affinity between a particular antigen-receptor complex and yields useful information about their relationship.

Experimental Procedure:

The objective of wafer fabrication was to create a planar substrate with aluminum filled patterns that lay flat with the surrounding surface. The process began with a two-layer resist stack being spun on fused silica wafers. The bottom layer was comprised of LOR3A liftoff resist, approximately 150 nm thick, and the top layer consisted of S1813 photoresist, approximately 1 μm thick. Micron-size features were patterned by contact lithography, using an ABM contact aligner with a seven second exposure. After development in MF321 for 30 seconds, the patterns were etched 150 nm deep by fluoroform/oxygen gas chemistry in an Oxford 80 etcher. Finally, 150 nm of aluminum was evaporated into the etched channels with a CHA evaporator. After wafer fabrication was complete, it was diced up into 14 mm × 14 mm squares. Pattern fidelity was verified by atomic force microscopy (AFM) after fabrication.

A supported lipid bilayer was constructed on top of each die via vesicle fusion, aided by calcium chloride. Shortwave UV radiation was shone through the bottom of the wafer and polymerized lipids not protected by the aluminum pattern. Afterwards, the diffusion coefficient of the hindered lipids in various regions of the die was determined by FRAP.

FRAP is a technique used to determine a diffusion constant for mobile, fluorescent substrates. A small region of the substrate is bleached by brief, intense exposure to radiation. The spot is then observed over a period of time and the diffusion of the photobleached lipids out of the spot and unbleached lipids into the region is noted. The rate of this diffusion is thus indicative of the overall diffusion coefficient for the membrane. The FRAP process employed was performed on an Olympus IX71 inverted microscope.

SLBs prepared on glass bottom dishes were examined on the microscope with 1% light intensity and photobleached with 100% intensity. Spot size was controlled by partially opening and closing the aperture on the microscope. This

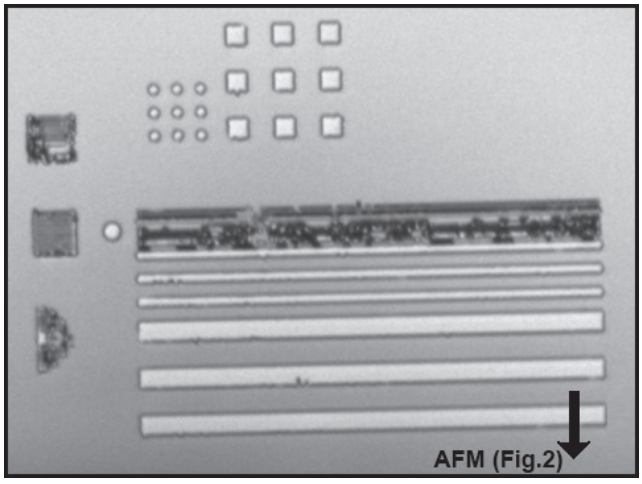

Figure 1: Micron-scale patterns coated in aluminum with edge roughness due to angled deposition.

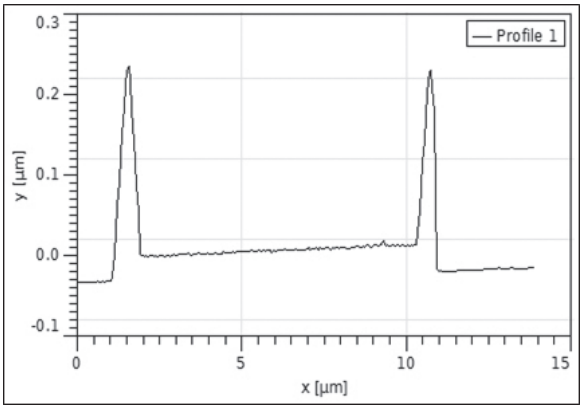

Figure 2: Profile scan of previous pattern generated from AFM.

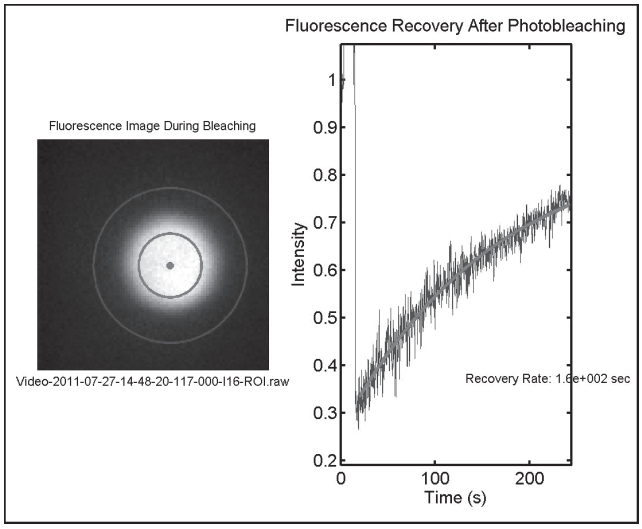

Figure 3: Image of photobleached region of lipids and resulting intensity curve during FRAP.

process was recorded on video and processed in MATLAB® to produce spot intensity versus time plots. The recovery time of the photobleached spot to half original intensity is proportional to the ability of the particular lipid composition to diffuse freely.

Results and Conclusions:

This technique has been established to the extent that it can be applied to more complex membrane models. Lipid mobility is, however, affected by surface roughness at the wafer-aluminum interface. The initial diffusion coefficients for SLBs of the same composition determined by this method have ranged from 180 seconds to 1200 seconds. Currently there is significant variation in the results for what should be equivalent samples. Increasing the proportion of fluorescent lipid in the membrane composition may improve contrast and offer more consistent results. This technique has much potential to profile lipid interactions in great detail after it has been honed and perfected.

Future Work:

Future work includes improvement of surface smoothness for more consistent lipid diffusion, the use of more complex lipid models that include receptors and fluorescently tagged antigens, and scaling the patterns down to the nanoscale.

Acknowledgements:

This work was made possible by funding from the NSF and by the REU program by the NNIN. Thanks to Dr. Christopher Kelly for continued support during the project, as well as Professor Harold Craighead and the Craighead Research Group. Thanks also go to the staff of the Cornell NanoScale Facility who assisted with the research and helped organize the REU program.